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Intracellular Signalling: The Language of the Chloroplast

Dispatch

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Chloroplasts emit signals that modulate nuclear gene expression, thereby ensuring the proper assembly of the photosynthetic apparatus. Recent studies have provided major new insights into one of these signalling mechanisms, and identified the chlorophyll precursor Mg-protoporphyrin IX as a key signalling molecule.

Chloroplasts, like mitochondria, evolved from a free-living prokaryotic organism that entered the eukaryotic lineage through endosymbiosis. During the course of their evolution, chloroplasts relinquished most of their genes to the nucleus, and so became subservient to the eukaryotic host. Today, more than 90% of the 3000 or so proteins present in chloroplasts are encoded in the nucleus, translated in the cytosol and imported into the organelle post-translationally [1,2]. The remainder are encoded and synthesized within the organelle itself by an endogenous genetic system. One of the consequences of this partitioning of genetic information is that processes which take place inside chloroplasts necessarily require input from two different compartments. For example, the photosynthetic complexes of the thylakoid membranes comprise core subunits encoded by the chloroplast genome, and peripheral subunits encoded by the nuclear genome. To ensure that these complexes are assembled in stoichiometric fashion, and to enable their rapid reorganization in response to changing environmental cues, the activities of the nuclear and chloroplast genomes must be closely coordinated through intracellular signalling.

The pre-eminence of the nucleus in this inter-organellar exchange is indisputable. Nevertheless, it is now well established that signals emanating from chloroplasts can have a profound effect on events in the nucleus [3]. Given the importance and complexity of processes such as photosynthesis, it is not surprising to learn that chloroplast signals are multifarious in nature [4,5]. For example, redox poise within the photosynthetic electron transfer chains, the accumulation of reactive oxygen species, and the perturbation of the chlorophyll biosynthesis are all known to influence nuclear gene expression. Of these, the signalling pathway related to chlorophyll biosynthesis was the first to be discovered, and is now best understood. In fact, two new papers [6,7] from the laboratory of Joanne Chory have shed significant light on how this signalling pathway functions.

About ten years ago, Chory and colleagues conducted a genetic screen for *Arabidopsis* mutants with defects in chloroplast-to-nucleus communication [8]. The rationale for the screen hinged on previous observations, from several different laboratories, that the transcription of nuclear genes encoding chloroplast proteins is strongly repressed if chloroplast development is blocked through photooxidative damage [9]. The herbicide norflurazon causes a deficiency in photoprotective carotenoid pigments, which in turn causes the complete photodestruction of the chloroplast interior, but leaves the rest of the cell intact. Under these circumstances, genes such as those encoding the light-harvesting chlorophyll *a*/*b*-binding proteins of photosystem II (*Lhcb*) are completely repressed. Susek *et al.* [8] fused an *Lhcb* promoter to a selectable marker gene, and then used the resulting chimera to screen for *Arabidopsis* mutants no longer able to repress *Lhcb* expression upon growth in the presence norflurazon. The mutants identified were referred to as *genomes uncoupled*, or *gun* mutants.

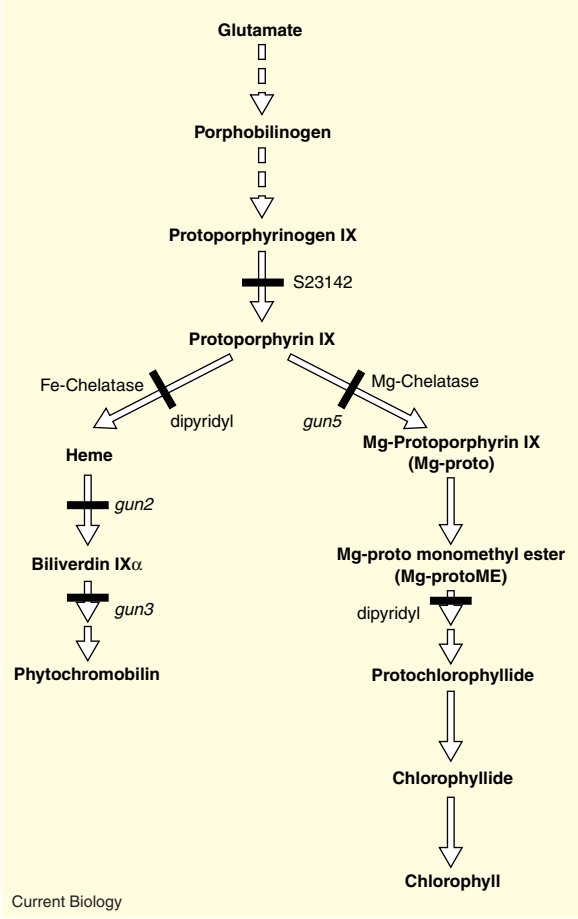

Figure 1. The tetrapyrrole biosynthetic pathway of chloroplasts.

Genetic lesions — *gun2*, *gun3* and *gun5* — and pharmacological agents — dipyridyl and S23142 — that disrupt defined steps in the pathway are indicated.

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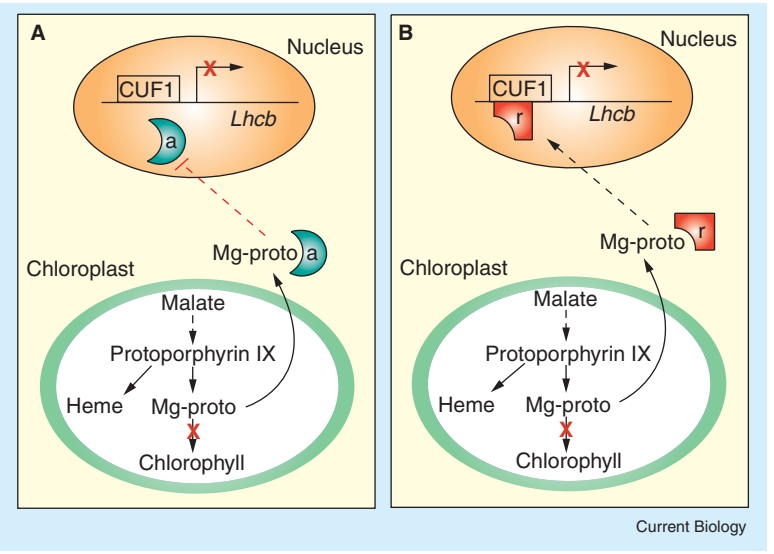

Figure 2. Two different models for Mg-proto signal transduction.

Certain conditions cause a build-up of Mg-proto in chloroplasts. Accumulated Mg-proto exits the chloroplast by passive diffusion or active transport, and then interacts with a regulatory protein. (A) Mg-proto may bind to an activator (a) and prevent its translocation to the nucleus or inhibit its binding at the CUF1 element of the *Lhcb* promoter. (B) Alternatively, Mg-proto may bind to a repressor (r) and promote its translocation to the nucleus or stimulate its binding at the CUF1 element. In all scenarios, transcription of the *Lhcb* gene is repressed.

Two years ago, Mochizuki *et al.* [10] demonstrated that three of these *gun* mutants carry lesions in the tetrapyrrole biosynthetic pathway of chloroplasts (Figure 1). The *gun2* and *gun3* mutations affect the heme branch of the pathway, and are presumed to affect flux through the chlorophyll branch as a result of feedback regulation. The *gun5* mutation lies in the gene coding for the H subunit of Mg-chelatase (ChlH), and affects the first step in the chlorophyll branch of the pathway. In agreement with earlier studies, these results strongly suggested a role for tetrapyrroles in chloroplast-to-nucleus communication. Unfortunately, the identity of the active signalling intermediate remained elusive.

This puzzle has now been solved. In a recent paper, Strand *et al.* [7] present evidence that the active compound is Mg-protoporphyrin IX (Mg-proto), the product of the Mg-chelatase reaction (Figure 1). A range of different experiments were used to constrain the identity of the signal to a single molecule. First of all, wild-type *Arabidopsis* plants were found to accumulate high levels of Mg-proto when grown on norflurazon; this accumulation response was partly or completely absent in the *gun2* and *gun5* mutants. Next, pharmacological agents that inhibit defined steps in the tetrapyrrole pathway — dipyridyl and S23142 — were used to predictably modulate the *gun2* and *gun5* phenotypes (Figure 1). Dipyridyl application restored normal *Lhcb* repression in norflurazon-treated *gun* plants — presumably by allowing Mg-proto to accumulate to sufficiently high levels — and S23142 application abrogated the restorative effect of dipyridyl. Finally, the effects of individual tetrapyrroles on *Lhcb* expression were assessed directly in feeding experiments using wild-type *Arabidopsis* protoplasts. In these experiments, only Mg-proto repressed gene expression; porphobilinogen, protoporphyrin IX and heme all had no effect.

These results are broadly in agreement with several earlier studies. For example, Johanningmeier and Howell [11] showed that dipyridyl treatment strongly represses *Lhcb* expression in the unicellular alga, *Chlamydomonas reinhardtii* — a treatment that a year earlier had been shown to cause massive accumulation of Mg-protoporphyrin IX monomethyl ester (Mg-protoME) [12]. Again, the application of inhibitors affecting earlier steps in the tetrapyrrole pathway abrogated the effect of dipyridyl treatment [11]. Similar inverse relationships between Mg-proto and Mg-protoME levels and *Lhcb* expression have since been observed in plants [13]. More recently, Beck and colleagues [14,15] demonstrated a direct effect of Mg-proto and Mg-protoME on nuclear gene expression in *Chlamydomonas*. A light-responsive gene encoding a chloroplast heat shock protein (*HSP70B*) could be induced in the dark by feeding either tetrapyrrole; protoporphyrin IX, protochlorophyllide and chlorophyllide all had no effect. Moreover, Mg-proto and Mg-protoME were shown to accumulate rapidly upon illumination, and a Mg-chelatase-deficient mutant no longer exhibited light-inducible *HSP70B* gene expression [14,15].

Having established that Mg-proto (and Mg-protoME) is a chloroplast signalling molecule, the next question is: how is it perceived? Strand *et al.* [7] propose that Mg-proto exits the chloroplast in order to modulate the activity and/or nuclear-translocation of a regulatory protein (Figure 2). This idea certainly seems feasible, as the reactions of chlorophyll biosynthesis between protoporphyrinogen IX and chlorophyllide all occur in the chloroplast envelope — from where intermediates could easily escape — and tetrapyrrole release from isolated chloroplasts is known to occur [16,17]. Interestingly, Beck and coworkers [15] found that, although protoporphyrin IX feeding causes Mg-proto/Mg-protoME accumulation in dark-grown *Chlamydomonas* cells, it does not cause induction of the *HSP70B* gene. They speculated that Mg-proto/Mg-protoME accumulated under these circumstances is present inside the chloroplast, and that its release to the cytosol — in a light-dependent process — is necessary in order for downstream signalling events to occur.

Exactly how Mg-proto might exit the chloroplast is not clear. Passive diffusion and active transport

models both seem feasible [16,17]. What is clear is that the final explanation must take account of another recent study from the Chory laboratory. Larkin *et al.* [6] cloned the *Arabidopsis GUN4* gene, and found that it encodes a novel chloroplast protein which associates with ChlH/GUN5 in a high molecular weight complex. Data indicate that the GUN4 protein stimulates activity of the Mg-chelatase enzyme, and that it is able to bind both protoporphyrin IX and Mg-proto; in other words, GUN4 stimulates synthesis of the chloroplast signal, and it also binds the signal. Because a significant proportion of the GUN4–GUN5 complex is associated with the chloroplast envelope membranes, it is tempting to speculate that GUN4 is required to promote Mg-proto release from chloroplasts.

Although the cytosolic steps of the postulated Mg-proto signalling pathway (Figure 2) are purely hypothetical, Strand *et al.* [7] clearly show that the trail ends at a G-box motif in the *Lhcb* promoter, CUF1 — a motif that was originally identified as a light-responsive element. Interestingly, several previous studies have shown that light and chloroplast signals converge at a common region within responsive promoters [18]. Similar G-box elements are present in many other chloroplast-responsive genes [7], and so it seems likely that a single chloroplast signal — Mg-proto — has the ability to control a large number of nuclear genes.

This notion takes further support from a recent DNA array study of nuclear genes encoding chloroplast proteins. Leister and co-workers [19] analysed the responses of ~3000 different *Arabidopsis* genes to 35 genetic and environmental conditions, and observed two diametrically opposed transcriptome responses: one in which the genes were predominantly up-regulated, and another in which the genes were predominantly down-regulated. This observation led to the proposal that there is a two-state 'master switch' — perhaps corresponding to the Mg-proto signal — which controls the expression of nucleus-encoded chloroplast genes. Superimposed over this 'master switch' control are additional levels of regulation, for fine-tuning purposes, as individual responses were characterized by a variable number of deviations from the overall trend [19].

The conclusive identification of such an important chloroplast signalling molecule marks a significant breakthrough in our understanding intracellular communication, and it signifies the end of an era of speculation stretching back some 20 years. That the signal turns out to be a tetrapyrrole is fascinating, as it has been known for many years that mitochondrial heme travels to the nucleus in order to modulate the expression of genes encoding mitochondrial proteins [20]. It will be interesting to see how far this mitochondrial parallel extends, as we seek to establish the means by which the Mg-proto signal is transduced to the nucleus.

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